A STUDY ON CHEMICAL CHARACTERIZATION, MUTAGENIC PROPERTIES AND CYTOTOXIC IMPACT OF PM_1 AEROSOLS AT SUB-URBAN AND URBAN SITES

Synopsis

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INTRODUCTION

Air pollution is one of the biggest environmental threats to human health, alongside climate change. Improving air quality can enhance climate change mitigation efforts. Assessments of ambient air pollution alone suggest hundreds of millions of healthy life years of life lost, with the greatest attributable disease burden seen in low and middle-income countries. Air pollution is the 4th leading risk factor for early death worldwide (SOGA, 2020). Exposure to air pollution is estimated to cause 7 million premature deaths every year. In children, air pollution could include reduced lung growth and function, respiratory infections, and aggravated asthma. In adults, ischaemic heart disease and stroke are the most common causes of premature death attributable to outdoor air pollution, and evidence is also emerging of other effects such as diabetes and neurodegenerative conditions. More the exposure to air pollution, the greater the health impact, particularly on individuals with chronic conditions (such as asthma, chronic obstructive pulmonary disease, and heart disease).

Clean air should be a fundamental human right and a necessary condition for healthy and productive societies (WHO, 2021). Impacts of climate change are "widespread and consequential" IPCC states that losses increase with greater warming, reinforcing the urgency to implement adaptation measures in parallel with mitigation strategies that reduce global emissions immediately and rapidly. The latest report by IPCC confirms that climate change isn't just a problem for future generations, but one that affects every one of us today (IPCC, 2021).

The burden of disease attributable to air pollution is on par with other major global health risks (WHO, 2021). PM is a complex mixture of extremely small particles and liquid droplets suspended in air and comprises of organic and inorganic chemical fractions. On the basis of size, they are categorised as PM_{10}, $PM_{2.5}$ and PM_1. PM_{10} are coarse particles with an aerodynamic diameter $\leq$10 μm which can reach upper respiratory tract and can by-pass the body's natural defenses in the nose. $PM_{2.5}$ are fine particulates with an aerodynamic diameter $\leq$2.5 μm that reach the lower respiratory tract and penetrate deeper into the lungs and can damage lung tissues. PM_1 are submicron particles with an aerodynamic diameter $\leq$1 μm; they can pass from lungs to blood stream and reach deeper into the vital organs with more serious ramifications. Around the globe, approximately 2.1 million deaths per annum are reported due to the

exposure to PM_1 aerosols (Caggiano et al., 2019). The intricacy of studying PM_1 increases when coupling its effects with climate change, as air quality and climate change have intertwined interactions. Particles originate from a variety of different sources both natural and anthropogenic like industrial emission, combustion processes, vehicular emissions, resuspension of dust, sea salt, fuel combustion, biomass burning, volcanic eruption and agricultural processes. Combustion-derived particles are largely found in the fine particle fraction or in the even smaller, submicron fractions.

PM carry toxic substances like Polycyclic aromatic hydrocarbons (PAHs), heavy metals, water-soluble fractions, and carbonaceous fractions. PAHs are environmental contaminants composed of fused aromatic benzene rings in linear, angular, or clustered manner. PAHs are known to be formed typically during incomplete combustion of organic matter. The major sources of PAHs at urban sites include: industrial processes, vehicular exhausts, waste incineration and domestic heating while at rural sites major sources include: combustion of biomass fuels like wood, crop residues, cow dung cakes, coal and coal briquettes. These pollutants are widely distributed in the atmosphere. In the atmosphere, PAHs may undergo both homogenous and heterogenous reactions to form nitro and oxy derivatives which are probably more carcinogenic and mutagenic (Jariyasopit., 2014). Even exposure to low doses of PAHs could induce early cell cycle arrest and cause DNA damage (Longhin et al., 2013). PAHs have received particular attention over last decades attributed to their toxic, mutagenic and carcinogenic properties and persistence in the environment (Biache et al., 2014). As a result, 16 PAHs are listed as priority pollutants by the United States Environmental Protection Agency (USEPA) and the World Health Organization (WHO) established an air quality standard of 1 ng/m^3 for Benzo[a]pyrene (Agudelo-Castañeda et al., 2017).

Heavy metals are also the essential toxic components of PM, and these non-biodegradable elements can severely affect human health via three routes of exposure - food intake, dermal contact and inhalation (Wei et al., 2017). These primarily come from a wide range of sources including road dust, agricultural dust, river beds, construction sites and mining operations. Heavy metals Cu, Cr, Fe, Ni, Pb, Zn, V, Cd, and Mn are the metals of the most immediate concern because of their detection frequency, also considered to be important airborne toxicants and are widely present in PM (Zhai et al., 2014). Exposure to these metals has been associated with cardiovascular diseases, cancer, and many other adverse health

effects as metals, can generate reactive oxygen species (ROS), which can subsequently damage DNA (Schilirò et al., 2015).

Water-soluble inorganic ions (WSII) are among the predominant chemical constituents of PM. They play a substantial role in the Earth's radiation balance and are related to atmospheric particle formation, growth, and transformation. WSII originate from several kinds of solid and liquid particles originating from natural and anthropogenic emission sources like vehicular emissions, biomass burning and also have potential to generate ROS which can damage DNA. Gao et al. (2015) demonstrated that 45.0 – 55.5% of atmospheric PM was attributed to WSII, and reported SO_4^{2-}, NO_3^- and NH_4^+ have a substantial effect on the atmosphere (Gao et al., 2015).

A mounting number of studies have examined the associations between PM exposure and adverse health effects (Liu et al., 2019a). When determining these associations, the size of PM plays an important role, since existing studies have demonstrated that the size of the PM can alter the PM-associated effects. Smaller PM may have a stronger impact due to the combined effects of its higher pulmonary deposition efficiency, larger surface area (Yin et al., 2020).

With the decreasing aerodynamic diameter of PM there is an enhancement of toxicity that was observed due to the increased surface area and chemical reactivity of the PM adsorbed species like trace metals (Chakraborty and Gupta, 2010). PM_1 can carry and deliver more redox-active organic compounds to the respiratory system than large particles, leading to a cascade of adverse effects related to oxidative stress and inflammation in the lungs and even in extrapulmonary sites (Delfino et al., 2010b). Moreover, smaller particle size indicates a more harmful effect on visibility, human health, and climate change. The fraction of PM deposited in human airways depends not only on the population characteristics but also on the size distribution (Deng et al., 2019).

Epidemiological studies have focused on $PM_{2.5}$ and PM_{10} while much less evidence is available for smaller particles, such as PM_1 and ultrafine particles. Due to the scarce monitoring of PM_1, only a few studies have found that exposure to PM_1 could trigger excess risk, including mortality (Yin et al., 2020), disease prevalence (Yang et al., 2019), hospital admissions (Chen et al., 2020b) and emergency department visits (Zhang et al., 2020). To better understand the dependence of the toxic potential of such particles on their composition and origin, additional toxicological studies are needed (Mohammadi

et al., 2016). The study of particulates is more important for their adverse health effects and their probable risk assessment. Although USEPA (2006) has assigned regulatory standards for $PM_{2.5}$ and PM_{10} (24 h $PM_{2.5}$ standard =35 μg/m^3 and annual $PM_{2.5}$ standard = 15 μg/m^3; 24 h PM_{10} standard of 150 μg/m^3), but till date no regulatory standards have been proposed for PM_1, highlighting a legislative gap. PM_1 pollution source in urban ambient air is largely low mass aerosol from motor vehicle combustion exhausts (Verma et al., 2017). The decrease in the aerodynamic diameter has led to a significant increase in the chemical reactivity of PM for heavy metal adsorption, enhancing the toxicity of those particulates (Wang et al., 2020). Slezakova et al. (2013) conducted a study and reported that PM_1 is more likely to reach deeper into the respiratory system and bloodstream carrying with it, more toxins from anthropogenic emissions and can harm the human respiratory and cardiovascular systems (Budisulistiorini et al., 2016). PM_1 can enter with more serious ramifications having severe health implications, With the advancement of our knowledge, the latest research findings reveal that it is not the $PM_{2.5}$ but PM_1 which has close relation with human health. PM_1 have large surface area due to which they have higher probability than larger particles to introduce adverse health effects (Slezakova et al., 2013). Studies have been conducted to obtain the characteristics of PM_1 (Cao et al., 2017), but there are scarce studies on their health risk assessment. To underpin the associations between PM_1 exposure and adverse health effects, it is necessary to accumulate more evidence from multiple studies. As a result, PM_1 is an important parameter to study in urban and sub-urban air pollution and is a serious health and environmental concern.

Literature Review

International Status

Atmospheric particulate matter has led to significant deterioration of the air quality in the past, and the present trends are continuing to add to the increasing pollution worldwide. A number of studies have been reported about the association between ambient particle (PM) and inauspicious health effects and variation in climate (Chakraborty and Gupta, 2010). In the past two decades majority of the studies focused on the measurement and chemical characterization (Boreddy and Kawamura, 2015). Mainka. (2021) carried out a study in Poland on PM_1 concentrations inside urban and rural kindergartens during the winter and spring seasons. In rural kindergartens PM_1 concentration were higher in winter and spring

and were reported 78.2 and 49.7 μg/m^3 respectively. In urban kindergartens PM_1 concentrations were reported 47.4 and 51.2 μg/m^3 during winter and spring, respectively. Health risks related to heavy metals (Cd, Cr, Cu, Fe, Mn, Ni, Pb, Sb, Se, Zn, and metalloid As) in the indoor dust samples were studied and the greatest concern was expressed for the concentration of carcinogenic Cd in PM_1 for the reported concentration of 6.7 to 9.7 ng/m^3 (Mainka., 2021). Caggiano et al. (2019) investigated PM_1 samples for mass concentration and sixteen elements in an anthropized area of international attention close to oil/gas pre-treatment plants in Agri Valley in Southern Italy. PM_1 mass concentrations were observed to be 3 to 16 μg/m^3 and elemental concentrations decreased in the order S > Ca > Na > K ≈ Mg ≈ Fe> Al > Li > Cr > Zn > Ti> Cu > Ni ≈ Mn > Pb ≈ Cd. The integrated carcinogenic risks were 3.45×10^{-5} and 1.38×10^{-4} respectively for children and adults indicating that attention should be paid for carcinogenic health effects. Nickel posed the highest non-carcinogenic risk for children through inhalation pathway (Caggiano et al., 2019). Haiying et al. (2021) evaluated the cytotoxic effects of $PM_{2.5}$ at the Shanxi University campus, Taiyuan, China, where freshly isolated alveolar macrophages (AMs) from Wistar rats were treated with extracted $PM_{2.5}$ They reported that $PM_{2.5}$ decreased cell viability and increased lactate dehydrogenase levels in AMs (Haiying et al., 2021). Massolo et al. (2002) conducted a study and found mutagenic potency (revertants/m^3) associated with particles less than 0.49 μm from urban areas of La Plata was about an order of magnitude higher than in particles in the range of 3.0 to 0.49 μm. Fine fractions from sites with an industrial burden were also found to exhibit high mutagenic potency. Mutagenic and cytotoxic effects were associated with very fine (< 0.49 μm) and fine (<1.5 μm) particle-bound compounds, corresponding to a higher content of PAHs (Massolo et al., 2002). Niu et al. (2021) conducted a study to determine the characteristics and cytotoxicity of organic aerosols (OA) in an urban roadside area in Hong Kong. The Non-refractory PM_1 was composed of organics (55 ± 15%), followed by sulfate (21 ± 9%), ammonium (13 ± 6%), nitrate (10 ± 6%) and chloride (1 ± 1%) (Niu et al., 2021). A study was done by Goudarzi et al. (2019) to investigate the chemical properties and in-vitro cytotoxic effects of $PM_{2.5}$ extracts. Water-soluble fraction and organic solvent-extractable components of the samples collected from the city of Ahvaz, Province, Iran, during the normal and dust storm days of the winter of 2016 were analysed and applied on the human lung epithelial cell line (A549). The chemical properties and the cytotoxicity were analysed. The results showed significantly

higher mean cytotoxicity in the samples of normal days than that of dust storm days. It could be due to the fact that the cells were exposed to the same amount of water-soluble extract, whether it be from a normal day or a dust storm day, and since the normal air of Ahvaz is severely affected by traffic and pollutants, its normal day samples showed stronger cytotoxic effect. The results showed that cytotoxic effect increased with the particulate concentration. The highest cytotoxicity levels observed at 62, 125, and 250 μg/mL concentrations after 24-h incubation were 17% (belonging to a dust storm day), 37% (belonging to a normal day), and 47% (belonging to a dust storm day), respectively (Goudarzi et al., 2019). Holland et al. (2017) have reported that finer aerosols may cause brain damage, vascular system inflammation or even lead to death (Holland et al., 2017). Elements such as, Ni, As, V, Pb, Cd, Cr, Cu, Mn and Zn are the important constituents of PM and are toxic in nature, therefore, it is important to know their composition and concentration in the ambient air (Gao et al., 2018). A study done at Dunkerque a French coastal industrial city were extensively characterized for physio-chemical properties, including inorganic and organic species. Mutagenicity assays using Ames test with TA98, TA102 and YG1041 Salmonella strains with or without S9 activation were performed on native PM sample and PM organic extracts and water-soluble fractions. BEAS-2B cell viability and cell proliferation were evaluated measuring lactate dehydrogenase release and mitochondrial dehydrogenase activity after exposure to PM and their extracts. The aim of study was to evaluate mutagenicity and cytotoxicity of a multi-influenced urban site PM, with or without the ultrafine fraction. PM induced cytotoxic effects were observed at relatively high doses while alteration of proliferation with low PM doses related to underlying mechanisms such as genotoxicity. This study shows how important it is to study ultrafine fraction whose mutagenic and cytotoxic effects appear to be more pronounced (Landkocz et al., 2017).

National Status

Ambient air pollution poses a grave concern for India. High concentration of PM has been documented over different regions of India that is characterized by extremely high levels of PAHs, WSII and metals**.** Chakraborty and Gupta (2010) carried out a study for chemical characterization of submicron **(**$PM_{1)}$ aerosols in Kanpur region and concentrations were measured at a sampling site inside the Indian Institute of Technology (IIT) Kanpur campus for 11 months from July, 2008–May, 2009. Average

PM_1 concentration was found to be highest (199 µg/m^3) during winter and lowest (31 µg/m^3) during monsoon season. Nitrate and sulfate were the two most predominant anionic species contributing to almost 80–90% of total anionic concentrations. Crustal elements (Ca, Mg, and Fe) were the main contributors within the total elemental concentrations. Overall, it was found that anions contributed up to 35% of the total PM_1 mass. Analysis revealed that secondary sources and vehicular emissions were the two main sources contributing to PM_1 mass with minor contributions from paved road dust and coal combustion sources (Chakrabarty and Gupta., 2010). Ernesto Reyes-Villegas et al. (2021) studied aerosol composition to compare Old Delhi (OD) and New Delhi (ND) over different seasons. No significant difference was observed in PM_1 concentrations between two sites, OD (142 ± 117 µg/ m^3) compared to ND (123 ± 71 µg/ m^3), for post-monsoon measurements. A wider variability was observed during pre-monsoon and monsoon showing PM_1 concentrations lower than 60 µg/m^3. There were no significant differences in PM_1 concentrations between the two sites, which suggests that controlling PM_1 levels above safe levels should be done at the local level for example considering the Delhi region, rather than considering controlling air pollution in OD or ND only. A seasonal variation in PM_1 composition was observed; SO_4^{2-} showed a high contribution over pre-monsoon and monsoon seasons, while NO_3^- and Cl^- had a higher contribution in winter and post-monsoon (Ernesto Reyes-Villegas et al., 2021). Manjunatha et al. (2020) selected following locations of Bengaluru city for the study: Basavanagudi (BAS), Domlur (DOM), Hosur road (HOS) and DC Halli (DCH). The mass concentrations of PM_1 samples collected varied from 20.16 to 68.64 µg /m^3. The highest mass concentration of 68.64 µg/ m^3 was observed for the location BAS and the lowest mass concentration of 20.16 µg/ m^3 was observed for the location DOM. The seasonal average mass concentration of PM_1 around Bengaluru for winter, summer, monsoon & post monsoon season during the entire study period was observed to be 47.60, 40.24, 30.85 and 38.76 µg/ m^3 respectively. The average 24 h mass concentrations of PM_1 in winter season that is in December month at BAS location was found to be higher than National Ambient Air Quality Standard limit of 60 µg/ m^3 for $PM_{2.5}$ however in January and February months mass concentration was found to be less than the standard limit (Manjunatha et al., 2020). Izhar et al. (2016) assessed health risk related exposure to toxic metals of PM_1 and concentrations of 13 heavy metals, adsorbed to submicron particles and these were experimentally examined in 2008-2009 but only 12

metals were found at detectable levels inside IIT Kanpur campus for all months excluding June and October. Results showed daily average PM_1 concentration is 102.46 ± 35.9 μg/m^3 and metal concentration followed the trend: Ca > Fe > Mg > Zn > Pb > Cu > Cr > Ni > Se > Cd > V > As. Cancer Risk for adults and children followed the same decreasing order, Cr (VI)>Cd > Ni > As > Pb. It was found to be higher than permissible limits (10^{-6}) for both adults and children (Izhar et al., 2016). Singh et al. (2015) conducted study on submicron particles, PM_1 were collected during the fog-dominated days during December 2013–January 2014. PM_1 values varied between 58.12 and 198.75 μg/m^3, and average mass concentration was 162.33 ± 38.25 μg/m^3 while total average concentration of particle-associated polycyclic aromatic hydrocarbon was 616.31 ± 30.31 ng/m^3. This is a signal for an alarming high pollution level at this site situated in the Indo-Gangetic Plain (IGP) (Singh et al., 2015). A study done by Singh and Gupta, (2016) reported health risk from 16 polycyclic aromatic hydrocarbons (PAHs) adsorbed on submicron particles and also reported their concentration, spatial distribution and possible sources during foggy days at Kanpur. They collected twenty-four urban foggy days samples in Kanpur, an urban centre in North India and most densely populated city in the Indo-Gangetic plain of India, and examined 16 PAHs. The mean concentration of PM_1 was found to be 160.16 ± 37.70 μg/m^3. ∑16PAHs concentrations were 529.17 ng/m^3 with a mean of 33.07 ng/m^3. The compounds of higher molecular weight added to 70.67% of ∑PAHs mass concentration in the foggy day's samples (Singh and Gupta., 2016).

Singh et al. (2015) detected Polycyclic Aromatic Hydrocarbons (PAHs) in PM_1 at Kanpur and reported 16 PAHs in which 14 are demarcated by USEPA as priority pollutants. Naphthalene showed lowest concentration of (4.04 ng/m^3) and fluoranthene showed highest concentration of 124.26 ng/m^3 (Singh et al., 2015). Singh and Gupta (2016) conducted a study on PAHs at a sub-urban site of Kanpur during foggy days on PM_1. The PAHs average mass concentration was 529.17±24.87 ng/m^3. The lowest concentration of PAHs was reported for naphthalene (3.76 ng/m^3) and the highest was for fluoranthene (97.53 ng/m^3) (Singh and Gupta., 2016). Massey et al. (2012) reported the concentration of PM_1 monitored from October-2007 to March-2009 indoors and outdoors at five roadside and five urban homes in Agra, India. PM_1 concentrations reported at roadside houses were 111 μg m^{-3} and 112 μg m^{-3} while at urban houses they were 99 μg m^{-3} and 104 μg m^{-3}. The concentration of PM_1 was

found to be highest in winter season due to increased human activities like biomass burning and more space heating in indoors and due to low windspeed and high humidity in outdoors in comparison with other seasons (Massey et al., 2012). A study conducted by Gupta et al. (2015) which shows the scenario of the prospective health risk of exposure to fine particulate matter and its composition at industry level. The indoor and outdoor sampling of fine particulate matter was conducted in residential site was a shoe industry in Agra city. The average concentration of PM was reported 30.11 ±10.29μg/m^3 for PM_1 at Industry-1, and 7.66 ±1.86 μg/m^3 for PM_1, at Industry-2. The concentration of PM_1 was found to be higher in Industry-1 in comparison to Industry-2 (Gupta et al., 2015). A study was conducted by Mangal et al. (2021) at Dayalbagh Educational Institute in Agra to determine concentration of crustal and trace elements. Mass concentration of PM_1 was 135.0±28.2 and 54.0±18.5 μg/m^3 during foggy and non-foggy period, respectively; crustal and trace elements were 13 and 4% during foggy and 11 and 3% in the non-foggy period (Mangal et al., 2021). In a study conducted by Agarwal et al. (2018) two Salmonella strains, TA98 and TA100, with or without liver S9 metabolic enzyme fraction were used to determine the mutagenic potential of particulate samples at different concentrations. Particulates emitted from diesel and gasoline engines showed higher mutagenicity, while those from CNG engine showed negligible mutagenicity compared to other test fuels and engine configurations. Polycyclic aromatic hydrocarbons (PAHs) adsorbed onto CNG engine particulates were also relatively fewer compared to those from equivalent diesel and gasoline engines indicating CNG is comparatively safer fuel compared to diesel and gasoline especially in developing countries (Agarwal et al., 2018). Agarwal et al. (2020) conducted a study at sub-urban and rural site of Agra to characterize WSII in terms of spatial, seasonal and formation characteristics. $PM_{2.5}$ mass concentrations were higher at sub-urban site (91.0 ± 50.8 μg/m^3) than at rural site (77.1 ± 48.6 μg/m^3). WSII contributed 50.0 and 45.8% of annual average $PM_{2.5}$ mass at both sites. The aerosols were ammonium rich and were therefore alkaline in nature. SO_4^{2-}, NO_3^- and NH_4^+ were the major contributors of WSII and their formation was favoured mainly in winter (Agarwal et al., 2020). Dubey et al. (2015) reported mutagenicity assessment of PAHs from Agra at a sub-urban site in $PM_{2.5}$ using *Salmonella typhimurium* strains TA98 and TA100 both with and without S9 mix which showed the direct acting mutagenicity obtained with TA98 ranged from 285 - 935 rev/m^3 with an average value of 594±1.4 rev/m^3. Direct mutagenic activity detected in the TA100 strains ranged from

343 to 1220 rev/m^3 (Average value 834±1.4 rev/m^3) without S9 and 467 to 1866 rev/m^3 (Average value 1195±1.5 rev/m^3) with S9 and a higher potential for base pair mutagenicity was reported as compared to frame shift mutagenicity due to greater mutagenic response towards TA100 (Dubey et al., 2015). Mangal et al. (2018) conducted a study on high loading of PM_1 mass during the episodic events i.e., Diwali and fog at the campus site of Dayalbagh Educational Institute, Agra. PM_1 was 6.8 times higher than the mass observed during normal days due to burning of firecrackers probably due to less dispersion of pollutants as a consequence of low mixing height, low temperature and high relative humidity (Mangal et al., 2018).

OBJECTIVES

Despite the health risks posed by PM_1, only few studies are reported in the literature. Hence, it is necessary to undertake studies on PM_1 including their chemical composition sources and health hazards to have a comprehensive knowledge of them. Thus, this study has been planned with the following objectives.

- Chemical characterization of submicron particles
- To study their mutagenic properties
- To evaluate the cytotoxic potential of submicron particles
- To carry out source apportionment of submicron particles using various models and statistical tools.

PROPOSED METHODOLOGY

Site Description

For the study sampling will be carried out at Dayalbagh and Sikandra in Agra. Dayalbagh is a sub-urban site. The population of Dayalbagh is about 2,830 (Census, 2011). Sikandra is an urban site surrounded by industrial sites; leather, chemical, packaging material, tyre, Exide battery industry, shoe factories (https://www.agraonline.in/city-guide/industries-in-agra) and the National Highway-19 is only 1.5 km away from sampling site. The population of Sikandra is about 1,02,493 (Census, 2011). The site has not been explored yet, so it is chosen as one of the sampling site for this study.

The city of Agra is situated in the north central region of India (27°10′ N, 78° 32′E) with two-thirds of its peripheral boundaries (SE, W and NW) being surrounded by the Thar desert of Rajasthan. The climate of Agra is hot and dry during the summer with a relative humidity of 25 to 40% and the

temperature ranging from 25 to 46° C, while the winter season features a cold weather with a relative humidity of 60 to 90% and low temperature ranging between 0.6 to 22°C. The annual rainfall in Agra is about 800 mm and the winds usually come from west and north-west directions.

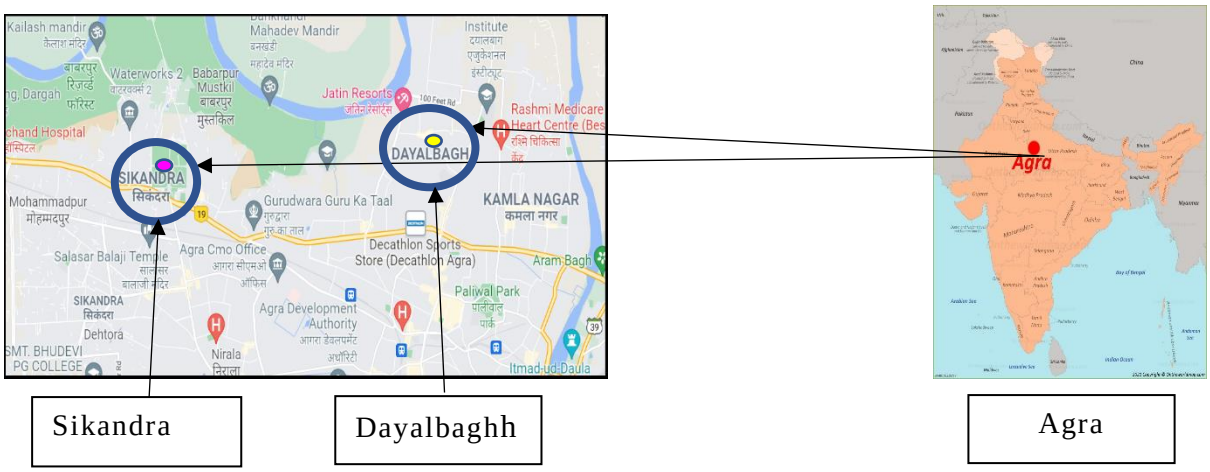

Figure1. Sampling sites

Sample collection and the measurement

For collection of PM_1 samples, high volume Sampler (Envirotech APM 577) will be used. It will be operated at a constant flow rate of 10 L/min for 24 hours and glass fibre filter paper of 47mm will be used for sample collection. For measurement of mass concentrations, the filters will be weighed before and after each sampling using a Mettler Microbalance. Sample will be collected weekly and simultaneously at both the sites. The samples will be wrapped in aluminium foil, kept in zip lock bags, and stored in a freezer at −4°C until the chemical analysis. Meteorological parameters like temperature, solar radiation, wind speed, wind direction, and relative humidity will be recorded by Polludrone (Oizom) real time air quality monitor for the Dayalbagh Site and these parameters will be taken from CPCB (https://app.cpcbccr.com/ccr/#/caaqm-dashboard-all/caaqm-landing/datadays) at Shastri Puram, Agra for the study at urban site, Sikandra.

The mass concentration of PM in the ambient air will be determined gravimetrically by the difference in the mass of the filter before and after the sampling the filter papers will be divided into four equal parts for further analysis as detailed below.

Analysis of Water- soluble Inorganic ions (WSII)

After sampling, one-fourth of the sample loaded filter paper will be extracted by sonicating with deionised water for 45 minutes. WSII will be determined using Dionex ICS 1100 Ion Chromatograph

system. The cation analysis will be performed using 20 mM methane sulphonic acid as an eluent for Na^+, K^+, Mg^{2+} and Ca^{2+}. The system will be equipped with guard column, analytical column and cation self-regenerating suppressor. The anion analysis will be performed using 6 mM NaOH as an eluent for Cl^-, NO_3^- and SO_4^2. The system will be equipped with guard column, analytical column and anion self-regenerating suppressor.

Analysis of PAHs

The one-fourth of the filter paper will be cut into small pieces and will be extracted using 150 ml Dichloromethane (DCM) by ultrasonication for 60 minutes. The extracts will be filtered by using filter papers and will be cleaned employing a silica gel column. The extracts will be combined and concentrated to 2 mL using a rotary evaporator. The samples will be stored in glass vials wrapped in aluminium foil at low temperature until analysis. PAHs will be analysed by Gas Chromatograph Mass Spectrometry (GC-MS).

Analysis of Metals

One-fourth of the sampled filter paper will be used for microwave-assisted acid digestion (Microwave Go Plus, Anton Paar) in 6 ml HNO_3 (69%). After digestion, the extract will be transferred to pre cleaned PTFE bottles and will be made up to 25 ml with deionised water. Concentration of metals will be quantitatively determined by inductively coupled plasma optical emission spectrophotometer (ICP-OES, Agilent).

Mutagenicity Assay

Mutagenicity assay will be conducted by the Ames test (Maron and Ames,1983) with *Salmonella typhimurium* TA98 and TA100 strains, respectively. The test will be performed with and without the presence of S9 fraction. The principle of this bacterial reverse mutation test is that it detects mutations which revert mutations present in the test strains and restore the functional capability of the bacteria.

Cytotoxic Potential

To evaluate the cytotoxic potential of submicron particle (PM_1), biological bioassays will be used. As per literature review, cytotoxic potential in PM can be assessed by (i) MTT assay and (ii) mitochondrial succinate dehydrogenase (MDH) activity and (iii) cell viability as evaluated by measurement of LDH in cell-free supernatants. The amount of released LDH can be used as an indicator of the cytotoxic effect

on cell walls. LDH assay is a colorimetric method, which is based on the conversion of yellow tetrazolium crystals by the succinate dehydrogenase and the formation of blue insoluble formazan crystals. This assay can be performed using a commercial LDH kit (Takara, Japan) (Alessandria et al., 2014). After the incubation of the cells the supernatants will be separated and then will be kept at room temperature for 30 min. Finally, the samples will be analysed by ELISA, and the cytotoxicity level will be calculated using the following equation:

Cytotoxicity% = exp. value – low control / high control – low control * 100

1) High Control: High control is obtained from the cells treated with Triton X-100 instead of particulate suspensions and represents the maximum concentration of LDH can be released from the cell (A549 cell + 100 μL of medium+100 μL of Triton X-100).

2) Low Control: Low control is obtained from the cells that are not treated with any sample (A549 + 200 μL of culture medium).

3) Background Control: Background control is obtained from 200 μL of medium without cell (Schilirò et al., 2015).

Tetrazolium compounds such as MTT, XTT and MTS are extensively used as convenient, non-radioactive methods for determining the number of viable cells in proliferation and cytotoxicity assays are based on the ability of viable cells to convert a soluble tetrazolium salt to a formazan product that is soluble in culture medium. Earlier studies demonstrated $PM_{2.5}$ induced cytotoxicity correlated decreasing cell viability MTT bioassay was used to determine the number of viable cells present. After removal of the supernatant, the dark blue formazan crystals dissolved in DMSO. The absorbance of the suspension was determined at 570 nm, and the measured values reported as a percentage of the control (Alessandria et al., 2014). The toxicity and viability calculation by using Equations (1) and (2)

Toxicity % = 1- (mean of OD of sample/mean OD of control) * 100 …. equation (1)

Viability % = 100-Toxicity % …. equation (2)

For data compilation and interpretation various statistical tools and software will be used.

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